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There are, under development, a number of new biological technologies that target the cleanup of contaminated hazardous waste sites. These technologies utilize specific organisms to degrade organic chemical contaminants to less toxic species. The attraction to this new area of treatment in the United States is the restrictions on waste burial, costs of conventional alternative technology, and the assumption that biological processes are more environmentally beging. Two areas of development that will be discussed in this paper are the use of a white rot fungus wood degrader for water and soil treatment and a pentachlorophenol degrader. The white rot based treatment is focused on the treatment of wood treating wastes, ie. creasote, pentachlorophenol and associated materials.

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PROMISING TECHNOLOGIES FOR THE BIOLOGICAL DETOXIFICATION OF HAZARDOUS WASTE

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ABSTRACT

A number of new biological technologies that target the cleanup of contaminated hazardous waste sites are under development. These technologies utilize specific organisms to degrade organic chemical contaminants to less toxic species. Two areas of development that will be discussed in this paper are the use of a white rot fungus wood degrader for water and soil treatment of wood treating wastes.

INTRODUCTION

Biological detoxification of hazardous waste is largely an underdeveloped technology. Recent publications have prompted a heightened interest in this area of research [1,2]. There are some twenty private biological control technology developers currently operating in the United States. Many of the treatment technologies are based on proprietary consortia of bacterial species. The use of bacterial consortia is based in part on the limitations of a single species to sufficiently degrade a specific waste. Different species are known to possess a variety of detoxification skills, apparently associated with the need to survive. Single bacterial species may not have the ability to convert a toxicant to carbon dioxide and water. Each species of the bacterial community plays a role in the overall degradation of the waste with the inadequacies of one offset by the abilities of another. The stability of such consortia is of some concern since there may be environmental effects that accentuate the selection of certain members over others, resulting in the loss or slowing of the degradation process.

The use of fungi to degrade waste materials has not been investigated to any extent until recently. Historically, filamentous fungi were to be avoided due to sewage processing problems. Also some of the fungi found in sewage are pathogenic. It may come as some surprise that a wood rotting fungus is now being investigated as a candidate organism for the treatment of hazardous waste.

Some of the more common substructures of lignin, 1,2-aryl diethers, alkyl sidechains, and connected aryl systems, resemble the chemical structures of many persistent organic compounds contaminating the environment. The remarkable similarity in structures suggested the value of investigating the applicability of a white rot fungus, Phanerochaete chrysosporium to the biodegradation of hazardous waste constituents [3]. The early findings of Bumpus [4] and Eaton [5], have significantly propelled the area of application of the white rot fungus, P. chrysosporium to the detoxification of hazardous waste constituents.

CHARACTERIZATION OF WOOD ROTTING FUNGI

There are some 1600 different wood rotting fungi known. These organisms are divided into three main categories: soft, white and brown rotting fungi [6]. The term white or brown refers to the characterization of residue left after infestation of a suitable host. The brown coloration characteristic of a brown rot, wood rotting fungus is attributable to portions of lignin incompletely degraded by the fungus and is to be contrasted with the white rot fungi that have more complete capabilities to degrade lignin.

P. chrysosporium is a filamentous, white, wood rotting fungus and has been typed to be a member of the Hymenomycetes subclass of Basidiomycetes [7]. Fungi are eukaryotic, ie. they possess a nuclear membrane and as microorganisms are considered to be plantlike forms, without chlorophyll having no photosynthetic abilities.

SUBSTRATE CYCLES OF WOOD ROTTING FUNGI

White rot fungi are primary wood degraders in nature [8]. All components of wood are degraded by these fungi with the naturally occurring polymers of cellulose and lignin forming the major sources of carbon to

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White rot fungi are primary wood degraders in nature [8]. All components of wood are degraded by these fungi with the naturally occurring polymers of cellulose and lignin forming the major sources of carbon to

assist fungal growth. Of the two general polymers, lignin, a structural component of wood, is by far the more difficult to degrade due to its composition as a heteropolymer formed from the cross linking of three precursor cinnamyl alcohols [9]. Of necessity, the fungus must be able to switch its ability to degrade these various polymers as the concentration of polymer varies with the composition of the wood. This ability for P. chrysosporium is activated by the absence of certain nutrients. Nitrogen deficiency is generally used to induce this secondary metabolic cycle of lignin utilization.

LIGNIN DEGRADING ENZYME SYSTEM

The structural features of lignin dictate unusual requirements of the biodegradation systems responsible for the initial attack on lignin. The 600-1000 k-dalton size range for lignin is far too large to enter the cells of microorganisms by known transport systems. An enzyme system permitting the microorganism to overcome this limitation must be extracellular, nonspecific and able to withstand deactiviation by proteases. Analogies with other biopolymer degrading systems fail since the enzymes of these other systems are highly specific and do not have protection against the action of proteases [9].

Among the more important reactions in lignin breakdown by P. chrysosporium are the cleavage of lignin alkyl side chains, ring demethylation, and ring cleavage. Hydrogen peroxide is consumed in this reaction by a peroxidative mechanism [10]. Stoichiometries of product formation as well as hydrogen peroxide and oxygen uptake are consistent with a radical pathway [11]. These results established the one-electron oxidative mechanism as the primary extracellular oxidative pathway for P. chrysosporium.

HAZARDOUS WASTE DEGRADATION STUDIES

Liquid phase studies have demonstrated that the white rot fungus can degrade a variety of structurally diverse organic compounds. Generally, chlorinated compounds were found to be mineralized more slowly than parent hydrocarbons. Pentachlorophenol, lindane, and chlordane are mineralized in liquid culture relatively fast whereas substrates such as 2,3,7,8-TCDD and pure tetrachloro polychlorinated biphenyl congeners react at a signif-

icantly lower rate [12]. The slow rate of degradation may not be a limitation of the fungal enzymes to oxidize the substrate but may be attributable to unfavorable transport limitations of substrates.

Fungal degradation of DDT has been extensively studied in liquid culture to determine the rate of substrate disappearance, metabolite identification, and mass balance analysis. The metabolites of fungal degradation of DDT indicate that the fungal pathway for the decomposition of DDT is different than that proposed for bacteria [13].

Hydrogen peroxide, a required reactant in the ligninase enzyme system can be produced by a number of enzymes in wood rotting fungi. Mineralization of organic substrates is dependent upon the ability of the fungus to generate H_2O_2 from atmospheric oxygen. In the extracellular enzyme mixture, there are a series of manganese enzymes thought to contribute this necessary oxygen conversion to the overall process [14,15].

LIFE CYCLE OF PHANEROCHAETE CHRYSOSPORIUM

To adequately harness the striking abilities of the wood rotting fungi, it is necessary to understand their life cycle. The Hymenomycetes fungi have a life cycle characterized by many structures formed during vegetative, sexual, and asexual reproductive phases. The thallus is the basic vegetative body of the fungus [6]. The vegetative phase is the longest and dominant growth phase. The highest concentration of extracellular enzymes are secreted during the vegetative phase. Eventually the tissues of the tertiary mycellium differentiate into fruiting bodies that are shed depending on environmental conditions. Asexual reproduction can occur anytime during the vegetative growth phase. P. chrysosporium produces asexual spores prolifically and at all stages of the life cycle [16].

ENZYME ACTIVITY STUDIES

It has been shown that P. chrysosporium produces at least ten extracellular hemoproteins (H1 through H10) and roughly half have ligninase activity [17]. The enzyme component designated H8 has been used by several researchers to characterize the ligninase activity. Depending on culture conditions, H8 can be displaced as the major component in favor

of other enzyme components. The extracellular hemoproteins have distinct amino acid sequences; hence they are separate gene products and not merely degradation products of a single precursor. The heme components H3-H5 have manganese peroxidase activity. This three component fraction catalyzes a hydrogen peroxide-dependent oxidation of Mn(II) to Mn(III) but lacks the specificity of H8 to cleave the alkyl side chains. The heterogeneity of the various extracellular proteins produced by P. chrysosporium points to possible functional differences that may be important to pollutant degradation. Attempts being made to uncover substrate specificities where information indicates that they may exist.

The ability of the ligninase H8 to oxidize polynuclear hydrocarbons has been related to the ionization potential of these compounds. The isolated enzyme H8 oxidizes the parent hydrocarbons to quinones that can be mutagenic by the Ames test [18]. When the parent hydrocarbons are substrates for the fungus, the mutagenic quinones do not accumulate but are metabolized to carbon dioxide.

Current work is directed to determine whether chlorinated aromatics are substrates for the extracellular ligninases [19,20]. Chlorinated phenols have been found to be very suitable substrates, and product identification is under way [21].

WATER TREATMENT SCALE UP

A water treatment process (MyCoR - Mycelial Color Removal) using P. chrysosporium is under investigation at the bench and is slated for scale up. The first application of this technology will be the treatment of waste derived from wood treating waste sites. In the United States these sites are a major problem awaiting cleanup. The total number of sites exceeds 700. The patented reactor is a specially designed rotating biological contactor that utilizes P. chrysosporium as the biological species for treatment [22]. Since the fungus does not have the same means to adhere to a surface as do bacterial species, the reactor design is modified to permit attachment of the mycelial mass to the plates. Recent results derived from the bench scale operation of this technology show that this reactor will degrade 250 ppm pentachlorophenol in water to 5 ppm in 8 hr [23]. Pink water associated with munitions production is

adequately treated. Degradation of 2,4,6-trinitrotoluene and 2,4-dinitrotoluene in concentrations up to 150 ppm occurs in 24 hr [23]. In both cases several sequential doses of the original concentration of contaminant are removed to the same extent. These results indicate that metabolism of substrate is occurring and not merely absorption onto the mycelial mat. Plans to scale up this technology in the next twelve months include pilot scale operations to treat surrogate waste. Once operational conditions are established, leachate derived from an actual wood treating site will be treated. Preliminary designs for this treatment at full scale call for parallel treatment trains.

SOIL TREATMENT RESEARCH [24,25]

The general success of solution biodegradation studies with the fungus stimulated speculation that this microorganism may be an appropriate candidate for the treatment of contaminated soils. At the outset of this research, P. chrysosporium was not known to inhabit the soil. Due to this general lack of knowledge of the habitat, a rather cautious research effort was engaged to determine the ability of the fungus to inhabit and thrive in the soil. Recent research has assessed the effects of selected soil types, temperatures, pH, and water potentials on the growth of the fungus in sterile and non-sterile soils. Three well characterized soils (topsoil and subsoil) were used in this work. Biomass accumulations as well as growth habit of P. chrysosporium were greatly influenced by soil type. Soil nitrogen content appears to be the primary factor responsible for differences in fungal growth in the three studied soils. Growth was strongly and positively correlated with nitrogen content. This factor, therefore, appears to play a major role in mediating the growth of the fungus in the soil, and is easily controlled by nitrogen supplementation.

Increasing the soil water potential from -1.5 MPa to 0.03 MPa resulted in greatly increased growth of P. chrysosporium. Research data suggest that fungal growth might benefit from soil water potentials greater than -0.03MPa. Water potential is another easily controlled soil factor [26].

Early work, indicated that P. chrysosporium did not grow well in non-sterile soils; this may be attributable in part due to ineffective competition with the indigenous microflora. These results were anticipated since the soil is not the normal habitat of P. chrysosporium. Lately, it has been found that growth within the soil can be accomplished through the use of larger quantities of inoculum.

The white rot fungus grows over a wide range of temperatures. Growth has been assessed from 10-39^{o}C. No growth was observed at 10^{o}C whereas growth significantly increases with temperature from 15 to 30^{o}C. No significant difference in growth was recorded between 30 to 39^{o}C. Soil temperatures under field conditions can be controlled by selecting the normal warm months and by soil solarization.

The application of the fungus to soil treatment is the remediation of wood treating sites. Target pollutants identified for treatment at these sites are pentachlorophenol and the major aromatic hydrocarbon contaminants found in creosote (napthalene, anthracene, and phenanthrene). Creosote has been extensively characterized and new substrates will be added to the mixture when deemed necessary [27]. The degradative ability of the fungus in the soil will be evaluated through the measurement of evolved carbon dioxide and material balances will be derived by the measurement of parent compound disappearance and the determination of identity and quantity of metabolic intermediates.

Future research in the soil application will include small scale treatment of selected pollutants at environmentally significant concentrations, the evaluation of amendments on primary and secondary metabolism, and the delivery of oxygen within the soil to the growing fungus. Ancillary investigations will include the development of analytical procedures to assay the fungal growth within the soil, the importance of soil sterilization to growth of the fungus, and inoculum development.

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